Comparison of Battlefield-Expedient Topical Antimicrobial Agents for the Prevention of Burn Wound Sepsis in a Rat Model

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Topical antimicrobial therapy has the potential to limit the mortality and morbidity of contaminated battlefield injuries. Many agents available are ill-suited for use on the battlefield; however, mafenide acetate solution (MAS) has known efficacy as a burn dressing adjunct, and previous work with mafenide as a direct chemotherapeutic has shown promise. A total of 71 male Sprague-Dawley rats underwent a 20% TBSA full-thickness scald. Wounds were inoculated with a solution containing 1×10^6 colony-forming units per milliliter of *Pseudomonas aeruginosa* 1244 (ATCC 27317). Treatments with 10% mafenide acetate cream (MAC), 5% MAS, 5% mafenide hydrochloride solution (MHS), and 4% chlorhexidine gluconate solution (CHG) were established. Agents were applied directly to the wound daily for 10 days. Animals were monitored for 21 days and euthanized if they manifested a moribund state as a result of sepsis. Survival to study completion in the negative control group (no treatment) was 25% (3/12). Survival in the positive control group (MAC) was 100%. None of the test agent groups demonstrated significant survival over the untreated controls; MAS resulted in 5/12 (42%) survival ($P = .67$), CHG in 4/12 (33%) survival ($P = 1.0$), and MHS resulted in 2/12 (17%) survival ($P = 1.0$). There were no significant differences in group weights on day 1. By day 6, all test agent groups were significantly underweight compared with the MAC group. This trend resolved as underweight animals died. We did not demonstrate significant prevention of wound sepsis with these agents as we used them. These techniques should not be substituted for established burn care. Aqueous direct topical antimicrobial agents have logistical advantages over creams and dressing soaks for field use, and the search for a battlefield-expedient agent for use at or near the point of wounding should continue. (J Burn Care Rehabil 2005;26:357–361)

Wounds sustained under battlefield conditions are considered to be contaminated, and their initial treatment should focus on decreasing this contamination and thus reducing the possibility of infection.[1] The role of wound infection and subsequent sepsis in adding to the morbidity and mortality of combat wounds is well-established.[1–5] Conditions on the modern battlefield often do not allow for the rapid evacuation of a casualty to surgical care and antibiotics and delay likely increases the chances of infection in contaminated wounds.[6] With the current threat of terrorist attacks creating battlefield-like conditions in civilian mass-casualty situations, the importance of developing frontline measures to prevent wound infection and sepsis is clear.

U.S. armed forces medical assets are increasingly involved in the care of injured noncombatants.[7,8] These patients, unlike rapidly evacuated U.S. casualties, undergo chronic inpatient treatment at U.S. facilities in theater. Caring for these individuals, although important, has the potential to strain a

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0273-8481/2005

DOI: 10.1097/01.BCR.0000170276.33207.B4

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Form Approved
OMB No. 0704-0188

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1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01 JUL 2005	**N/A**	**-**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Comparison of battlefield-expedient topical antimicrobial agents for the prevention of burn wound sepsis in a rat model	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
Kauvar D. S., Acheson E., Reeder J., Roll K., Baer D. G.,	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
United States Army Institute of Surgical Research, JBSA Fort Sam houston, TX 78234	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release, distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a REPORT	b ABSTRACT	c THIS PAGE			
unclassified	**unclassified**	**unclassified**	**SAR**	**5**	

facility's ability to provide ideal infection prophylaxis and treatment using in-theater assets. Traditional methods for prophylaxis and treatment of wound infections, such as dressing soaks and burn cream application, are time and resource consuming and are not suited for the conditions in modern field hospitals.

Despite experience with many topical antimicrobial agents and their proven efficacy in decreasing wound contamination and infection, combat medics and civilian first responders are not equipped with any such agents. This is, in part, the result of the fact that the most effective antimicrobials such as mafenide and silver sulfadiazine creams are too cumbersome for use in the field. We undertook this study using an established model of wound contamination and infection to test the effectiveness of field-expedient topical antimicrobial agents used in a novel fashion in the prevention of wound sepsis.

MATERIALS AND METHODS

After approval from our institution's animal care and use committee, 71 adult male Sprague-Dawley rats weighing 300 ± 15 g were anesthetized with isoflurane, shaved over the dorsum, and administered a standardized 20% TBSA full-thickness scald injury. One hour after scalding, wounds were inoculated with 1 ml of a solution containing 1×10^6 colony-forming units per milliliter of *Pseudomonas aeruginosa* 1244 (ATCC 27317) in 0.9% saline using a micropipette and cotton-tipped applicator. The applicator was kept saturated with the contaminated solution as it was wiped over the entire burned skin surface.

One hour after inoculation, treatment with four topical antimicrobial agents was initiated. The agents were as follows: 10% mafenide acetate cream (MAC-Sulfamylon® cream, Bertek Pharmaceuticals, Morgantown WV); 5% mafenide acetate solution (MAS–Sulfamylon® for Topical Solution, Bertek Pharmaceuticals, Morgantown WV); 5% mafenide hydrochloride solution (MHS, Sigma, St. Louis, MO), and 4% chlorhexidine digluconate solution (CHG, Sigma, St. Louis, MO). All treatments were stored before use in accordance with the manufacturer's directions (MAC, MAS, and MHS at room temperature, CHG at 4°C). Solutions were reconstituted and diluted to the appropriate concentration in sterile water. Agents were applied once daily for 10 days directly to the burn wound, covering the entire injury. MAC was applied per package directions to a thickness of 1/16th inch with a sterile tongue depressor. In previous studies at this institution, 1.5 ml was found to be the volume of solution adequate to cover the wounded surface in this model without excess. This amount of each of the test solutions was applied using a micropipette and cotton-tipped applicators. The applicator was saturated with solution and kept continuously moist as it was rolled over the entire wounded surface. There were no additional dressings.

Animals were returned to their cages and allowed food and water ad libitum. To assess the progression of infection, we weighed and evaluated them for physiologic and behavioral signs of sepsis daily for 21 days. Euthanasia was performed if animals suffered a decrease in body temperature greater than 4°C from baseline, lacked fecal output, or manifested a moribund state resulting in the inability to reach food or water. All surviving animals were euthanized on day 21.

RESULTS

Survival in the scald only (noninoculated study control) and MAC (positive experimental control) groups was universal. The study's primary endpoint was survival to study completion. None of the test agent groups demonstrated significant survival over the untreated group (Table 1). A total of 79% (27/34) of deaths occurred after the completion of treat-

Table 1. Characteristics of the study groups

	Scald	MAC	UNT	MAS	MHS	CHG
Number of animals	12	11	12	12	12	12
Avg initial weight (g)	291	305	301	303	302	299
Overall survival (%)	12/12 (100)	12/12 (100)	3/12 (25)	5/12 (42)	2/12 (17)	4/12 (33)
P compared with UNT*	N/A	N/A	NS	NS	NS	NS
Avg survival (days)	21	21	14	16	14	16

Scald, scald only (study control—not inoculated); *UNT*, untreated group (negative control-inoculated); *MAC*, 10% mafenide acetate cream (positive control); *MAS*, 5% mafenide acetate solution; *MHS*, 5% mafenide hydrochloride solution; *CHG*, 4% chlorhexidine gluconate.

* *P* for group survival at the conclusion of the study utilizing the Kaplan–Meier statistic. Considered significant at $P < .05$.

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ments, with 26 of these deaths between days 11 and 17, and only 21% (7/34) of deaths occurring during the treatment phase (Figure 1).

There were no significant differences between any group's average weight on day 1 or day 21. The positive and study control animals demonstrated consistent average weight gain from the third day after wounding until study completion. By day 6, all of the test agent groups were significantly underweight as compared with the positive and study controls. From day 7 onward, average weights increased in the test agent groups as moribund animals died (Figure 2).

DISCUSSION

The Walker–Mason burn model was developed at our institution and has proven to be an invaluable animal model in the study of thermal injury wound contamination, infection, and sepsis.[9–11] We have used this model in the search for a safe, effective, battlefield-expedient topical antimicrobial agent. We used established antimicrobial solutions in a novel fashion, applying them directly to the freshly contaminated wound surface without surgical débridment or antibiotic administration. None of the tested agents when used in this fashion significantly prevented mortality from wound sepsis.

We applied agents regularly for the initial 10 days after inoculation, during which time the animals are most susceptible to the development of wound sepsis in this model. The nature of this model of wound sepsis dictates that if agents are to have efficacy in the prevention of sepsis from a contaminated wound, they will demonstrate this with their use in the early period of the experiment, leading to survival to the end of the observation period, as seen in the MAC-treated positive control group. In similar experiments at our institution, this group is anticipated to produce 100% survival and is intended as a "gold standard" positive control. This particular experiment was designed for the primary comparison to be made between overall survival in the treatment groups vs the inoculated but untreated negative control group. Although most treated animals died after the discontinuation of treatments, they likely developed systemic infection during the treatment phase. This hypothesis is supported by the fact that the survival curve of the treatment groups is similar to that of the untreated negative controls.

We inoculated with a single strain of *Pseudomonas aeruginosa* known to be virulent in this model. How-

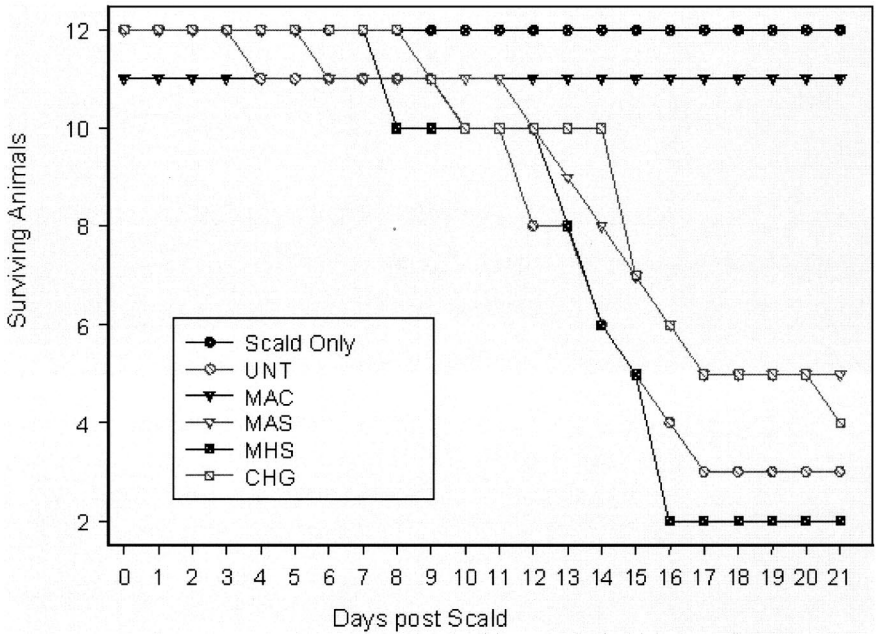

Figure 1. Kaplan–Meier survival curve for the study groups. The primary endpoint of the study was survival to study completion at day 21 vs inoculated, untreated negative control animals. No significant differences exist between any of the experimental groups and the untreated group at the conclusion of the study. The appearance of the treatment groups' survival curves is similar to that of the negative control's curve. *Scald Only,* scald only (study control—not inoculated); *UNT,* untreated group (negative control—inoculated); *MAC,* 10% mafenide acetate cream (positive control); *MAS,* 5% mafenide acetate solution; *MHS,* 5% mafenide hydrochloride solution; *CHG,* 4% chlorhexidine gluconate.

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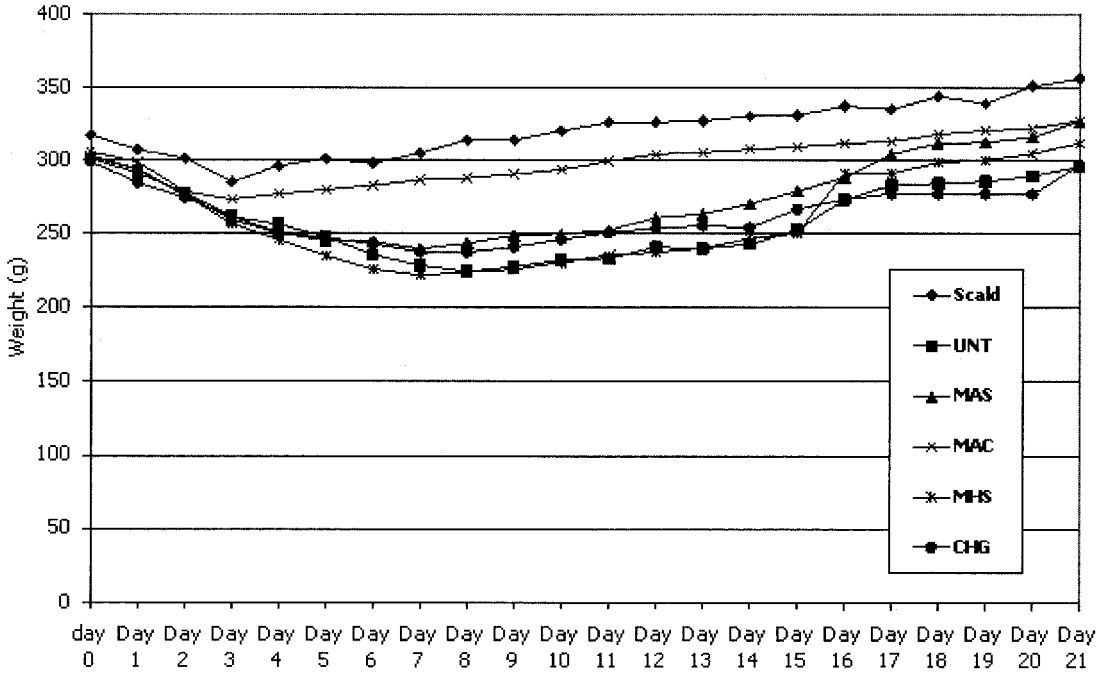

Figure 2. Group mean daily weights. No differences exist between group mean weights at the initiation and conclusion of the study. The inoculated, untreated negative control group and all treatment groups mean weights are significantly lower than the 10% mafenide cream-positive control group's at day 6. This trend resolves as underweight animals died throughout the observation period. The appearance of the treatment groups' mean weight curves is similar to that of the negative control's curve. *Scald,* scald only (study control—not inoculated); *UNT,* untreated group (negative control—inoculated); *MAC,* 10% mafenide acetate cream (positive control); *MAS,* 5% mafenide acetate solution; *MHS,* 5% mafenide hydrochloride solution; *CHG,* 4% chlorhexidine gluconate.

ever, given mafenide's known in vitro resistance patterns and the correlation between these and in vivo resistance patterns in this model, it is reasonable to anticipate that similar results would be obtained with other virulent strains as well.[12]

MAS has been approved by the U.S. Food and Drug Administration for use on autografted wounds and frequently is used as a dressing soak over débrided but not yet grafted burn wounds. There is no currently approved indication for use over fresh, non-débrided wounds. The results of our study suggest that mafenide solution would fail to be an effective field-expedient topical antimicrobial for use at the point of wounding and that it would lack efficacy if not used as a dressing soak following débridment. However, MAS is an established antimicrobial agent with proven clinical efficacy in inpatient burn care when used as a dressing adjunct. It is available as a powder and is reconstitutable in water, which provides logistical advantages for its use in the field. Its current clinical use requires frequent attention to reapplication because dressings dry out. However, this type of treatment, is time- and resource-intensive and is incompatible with the efficient provision wound care in current field hospitals.

The search for a topical antimicrobial agent for use on fresh combat wounds is not new. Clinical and animal research conducted by military surgeons during the Vietnam War attempted to identify an antibiotic topical spray that could be applied to fresh, contaminated war wounds and prevent local and systemic infectious complications of such injuries.[13,14] Despite promising early results, rapid surgical débridment remains the mainstay of infectious prophylaxis in combat wounds, and there is no widely advocated topical therapy.[15] The direct application of mafenide solution has been strongly advocated as a potential technique to serve as a field-expedient topical antimicrobial for the initial care of combat wounds.[16] Mafenide hydrochloride spray was used with some apparent success during the Vietnam War in an in-theater burn unit as an expedient antimicrobial agent during "chronic mass casualty" circumstances; however, this application was never validated in a controlled trial.[17] In 1970, Mendelson reported on a series of experiments in goats investigating the use of mafenide acetate and hydrochloride sprays applied via an atomizer twice daily for 14 days in a model of complex tissue injury (though not thermal injury) contaminated

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with *Clostridium perfringens*. In these experiments, 10% mafenide hydrochloride spray resulted in a survival advantage over both untreated control animals and animals treated with mafenide ointment.

Despite the efficacy of MAS as a burn dressing adjunct for débrided and grafted wounds and promising early work with mafenide hydrochloride as a direct chemotherapeutic in contaminated tissue models, we were unable to demonstrate significant prevention of wound sepsis with these compounds. Our use differs from previous animal models in the use of solutions directly on nondébrided thermal injuries. A possible reason for this failure of mafenide to prevent wound sepsis when applied directly to the freshly contaminated burn wound is the lack of time that the compounds were in contact with the wound surface. Although the specific mechanism of the antibacterial activity of mafenide is unknown, it is thought that the penetration of devitalized tissue by the agent over time contributes to the efficacy of MAC and solution dressing soaks.[18]

Chlorhexidine is a well-studied antimicrobial agent in burn treatment. It has shown antimicrobial efficacy in vitro when prepared as a solution and as a cream.[19,20] In addition, chlorhexidine in combination with silver sulfadiazine cream has demonstrated efficacy in the prophylactic antibacterial treatment of inoculated rat burns.[21,22] Chlorhexidine solution has not been previously tested as a direct antimicrobial agent for the prophylaxis of the contaminated fresh burn wound. We found it to be unsuitable for use in this manner.

We used these established agents in a novel fashion, applying them directly to the contaminated wound in the manner that they would be used on the battlefield by frontline providers. The ability to provide topical antimicrobial protection at or near the point of wounding has the potential to limit the morbidity and mortality of combat wounds. The search for a safe and effective topical antimicrobial agent for battlefield and mass-casualty applications should continue. The logistical characteristics of candidate agents are important considerations. The ideal agent will be light and easily transported, reconstitutable with available water, resistant to extremes of environmental conditions, and simple enough to use that a casualty could apply it themselves.

ACKNOWLEDGMENTS

The authors wish to acknowledge the contributions of Mr. Aldo Reyes, Mr. Charles Guymon, and the Veterinary Services and Support Branch of the U.S. Army Institute of Surgical Research for their contributions to this experiment.

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